Freshwater Quality Monitoring Protocol
San Francisco Area Network

Standard Operating Procedure (SOP) # 6

FIELD METHODS FOR SAMPLING FECAL INDICATOR BACTERIA

Version 1.01

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REVISION HISTORY LOG

Prev. Version #	Revision Date	Author	Changes Made	Reason for Change	New Version #
1.00	8/5/05	M. Cooprider	Minor edits	Preparation for formal peer review	1.01

Only changes in this SOP will be logged. "Version numbers increase incrementally by hundredths (e.g. version 1.01, version 1.02, …etc) for minor changes. Major revisions should be designated with the next whole number (e.g., version 2.0, 3.0, 4.0 …). Record the previous version number, date of revision, author of the revision, identify paragraphs and pages where changes are made, and the reason for making the changes along with the new version number" (Peitz et al, 2002).

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TABLE OF CONTENTS

LIST OF ACRONYMS USED

APHA	American Public Health Association
AWWA	American Water Works Association
EPA	Environmental Protection Agency
FDA	Food and Drug Administration
FIB	Fecal Indicator Bacteria
GOGA	Golden Gate National Recreation Area
I&M	Inventory and Monitoring
JOMU	John Muir National Historic Site
MPN	Most Probable Number
NAWQA	National Ambient Water Quality Assessment
PINN	Pinnacles National Monument
PORE	Point Reyes National Seashore
RWQCB	Regional Water Quality Control Board
SFAN	San Francisco Bay Area Network
SOP	Standard Operating Procedure
SWAMP	Surface Water Ambient Monitoring Program
TMDL	Total Maximum Daily Load
USGS	United States Geological Survey
WEF	Water Environment Federation

ACKNOWLEDGEMENTS

Other protocols and guidelines for followed during the development of this SOP. Many thanks are extended to the authors of these documents:

Puckett, M. 2002. Quality Assurance Management Plan for the State of California's Surface Water Ambient Monitoring Program ("SWAMP"). California Department of Fish and Game, Monterey, CA. Prepared for the State Water Resources Control Board, Sacramento, CA. 145 pages plus Appendices.

Myers, D.N, November 2003, Fecal indicator bacteria: U.S. Geological Survey Techniques of Water-Resources Investigations, book 9, chap. A7, 3rd edition, Section 7.1 accessed __*date*__ at http://pubs.water.usgs.gov/twri9A7/ (Chapter sections are cited by author and date.)

Wilde, F.D., Radtke, D.B., Gibs, Jacob, and Iwatsubo, R.T., eds., September 1999, Collection of water samples: U.S. Geological Survey Techniques of Water-Resources Investigations, book 9, chap. A4, accessed __*date*__ at http://pubs.water.usgs.gov/twri9A4/

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1.0 SCOPE AND APPLICATION

1.1 Fecal Indicator Bacteria and Their Relation to Water Quality

Wastes from warm-blooded animals harbor numerous intestinal bacteria that can be pathogenic to humans and other animals. These wastes can enter surface waters via surface runoff, groundwater flow, direct access of animals to a creek, leaky septic systems, and leaky sewer pipes. Fecal indicator bacteria (FIB) are used as indicators of the possible presence of pathogenic bacteria that may occur in wastes. Indicator species, as opposed to the pathogenic bacteria themselves, are enumerated because they are easier and safer to work with in the laboratory.

The most commonly used fecal indicator organisms or groups include total coliforms, fecal coliforms, fecal streptococcus, *E.coli*, and Enterococcus. The number of fecal coliforms is often highly correlated with other indicator species or groups including *E .coli* and *Enterococcus* though they cannot definitively be used interchangeably (Noble et al., 2000). Unlike total coliforms, *E.coli* and fecal coliforms are more frequently found in mammalian or avian intestines. Therefore, they are more pertinent as indicators of fecal material and associated pathogens in water. Total coliforms, including species in the genera *Klebsiella*, *Enterobacter*, and *Escherichia,* are indicators of all members of the *Enterobacteriaceae* family. However, some members of this family do not pose threats to human health so it is not particularly useful to know their occurrence (Turco, 1995). Another coliform, *Enterobacter aerogenes* is also frequently isolated from soils regardless of the presence of animal wastes. Total coliforms are ubiquitous in nature (Baxter-Potter and Gilliland, 1988). Numbers of total coliforms and other FIB in "natural" surface waters are listed in Table 1.

Table 1. Ranges of fecal indicator bacteria typically found in uncontaminated surface water and contaminated surface water (from Table 7.1-1 in the USGS National Field Manual)

Bacterial Group	Uncontaminated surface water	Fecal-contaminated surface water
	colonies/100mL	
Total coliform	<1 to 80,000	1,200- > 4,000,000
Fecal coliform	<1 to 5,000	200 to > 2,000,000
Escherichia coli	<1 to 576	126 to > 2,000,000
Fecal streptococococcus	<1 to 1,000	400 to > 1,000,000
Enterococcus	<1 to 100	100 to > 1,000,000
Clostridium perfringens	<1 to 100	100 to > 10,000

Fecal coliforms indicate the presence of feces that may contain pathogens in the genera *Salmonella*, *Mycobacterium*, *Leptospira*, *Clostridium*, and *Bacillus*, foot-and-mouth disease virus, entroviruses, and helminths (parasitic worms) (Reddy et al, 1981). *E.coli* is

rarely pathogenic. However, some pathogenic strains of E. coli can cause gastroenteritis, diarrhea, colitis, or dysentery. Strain O157:H7 can be fatal to infants, older adults, and individuals with compromised immune systems.

1.2 Water Quality Standards for Fecal Indicator Bacteria

Water quality standards for FIB have been established to protect human health. The San Francisco Bay Regional Water Quality Control Board (Regional Board) sets numeric and narrative objectives for water quality (Regional Water Quality Control Board, 1995). Table 2 shows water quality objectives for the primary beneficial uses of SFAN water bodies.

Table 2. General numeric objectives for select beneficial uses in surface waters in the San Francisco Bay Area.

Beneficial Use	**Fecal Coliform (MPN/100mL)**	**Total Coliform (MPN/100mL)**
Contact recreation	Log mean < 200 90^{th} percentile < 400	Median < 240 No sample > 10,000
Non-contact recreation	Mean < 2000 90^{th} percentile < 4000	
Shellfish harvesting	Median < 14 90^{th} percentile < 43	Median < 70 90^{th} percentile < 230

For the purposes of FIB monitoring, waters can be divided into three broad categories of beneficial uses including recreational, shellfish-growing waters, and ambient waters. Recreational waters are used for "contact recreation" such as swimming or kayaking. Ambient waters are used for "non-contact recreation" such as hiking and picnicking.

E.coli and *Enterococci* are the preferred indicators for contact recreational monitoring since they have greater survival in marine waters. Therefore, they are better indicators of swimming-related gastroenteritis in marine and freshwaters than total coliforms, fecal coliforms, and fecal streptococci. However, the I&M program will not be monitoring recreational waters which primarily include marine waters within PORE and GOGA.

1.3 Fecal Indicator Bacteria Levels in SFAN Waters

The UC Berkeley report *A Review of Water Quality Monitoring Programs in the National Parks in Central Coastal California* (Stafford and Horne, 2004) contains additional background information related to fecal indicator bacteria. Ranges in fecal indicator bacteria concentrations in SFAN parks are listed in Table 2. Additional information about FIB levels and sources in SFAN waters is included in the SFAN Preliminary Water Quality Status Report (Cooprider, 2004).

Table 3. Range in Fecal Indicator Bacteria* in SFAN parks (MPN/100mL) based on land use

Park	Overall Land Use (overall range)	Wilderness (mean range)	Grazed (mean range)	Dairy (mean range)
GOGA	2 to 300,000			
JOMU	17 to 900			
PINN	3 to 440 (E.coli)			
PORE †		17 to 540	1,000 to 46,000	2,400 to 710,000

* Fecal coliforms unless otherwise indicated
† The overall range in fecal coliforns at PORE was < 200 to > 1 million

Several water bodies within SFAN have elevated levels of FIB. A few of these water bodies are on the Clean Water Act Section 303d list due to impairment by fecal coliforms. Sources of fecal bacteria within SFAN include agriculture (dairy and beef cattle ranching and vegetable farming), residential areas (septic systems), and recreational land uses (equestrian operations and dog walking). Other streams are impaired due to their urban location and proximity to sewer pipes.

1.4 Tomales Bay Pathogen Total Maximum Daily Load (TMDL) Project

The San Francisco Bay RWQCB has identified Tomales Bay (PORE/GOGA) and its tributaries (Lagunitas Creek and Walker Creek) as impaired by fecal coliform. Health concerns have arisen due to contamination of shellfish with pathogenic bacteria. SFAN staff have collaborated with the RWQCB regarding monitoring of indicator bacteria in Olema Creek a tributary to Lagunitas Creek. The RWQCB recently completed a final Total Maximum Daily Load (TMDL) project report for pathogens in Tomales Bay (RWQCB, 2004). Implementation of monitoring for the Tomales Bay Pathogen TMDL program includes monthly monitoring plus five consecutive weeks of monitoring during the winter and summer in order to obtain a geometric mean.

The TMDL Implementation Plan is focused on attaining the water quality standard for shellfish harvesting areas of 14 MPN/100 mL for fecal coliforms. The Food and Drug Administration (FDA) regulates shellfish harvesting areas based on fecal coliforms. Therefore, although other FIB can be used to determine the presence of pathogenic bacteria, PORE is required to monitor fecal coliforms as part of the TMDL Implementation strategy. In order to maintain consistency, fecal coliforms, as opposed to other FIB will be monitored in the other SFAN streams as well.

1.5 FIB Sampling and Analysis Methods Overview

The definition of the coliform group has traditionally been based on the detection method used (lactose fermentation). A common technique for determining lactose fermentation involves inoculating multiple test tubes with the water sample. Results of the

examination of replicate tubes and dilutions are reported in terms of the Most Probable Number (MPN) of organisms present. This number is based on certain probability formulas and is an estimate coliform density in the sample (APHA, AWWA, WEF, 1998). Results are reported in units of MPN/100mL. Most Probable Number tests for total and fecal coliforms and *E. coli* usually result in greater recovery of microorganisms than other techniques such as membrane filtration (Myers, 2003).

Bacteria are often associated with sediment particles. Therefore, USGS recommends that depth-integrated sampling be conducted for bacteria sampling in the same way that it is conducted for sediment. However, the San Francisco Bay RWQCB does not use depth-integrated sampling for bacteria or nutrient TMDL monitoring (Peter Krottje, personal communication). The RWQCB's Surface Water Ambient Monitoring Program (SWAMP) also does not collect depth-integrated samples for bacteria. Regardless, in many cases with SFAN streams, there is not sufficient depth, except during storm events, to obtain a meaningful depth-integrated sample. In order to maintain consistency at all of the sites and throughout the sampling season, it is best to obtain a "grab" or "hand-dipped' sample.

This field SOP will focus on field sample collection including sterile technique to avoid contaminating a sample and location of sample in the water column. Details of sample bottle labeling, storage, and transport to laboratories (including chain of custody forms) will also be discussed. Analysis will be conducted by an analytical laboratory. Fecal coliform samples will be analyzed at an EPA approved laboratory using the SM 9221E Multiple Tube Technique (Most Probable Number) in "Standard Methods for the Examination of Water and Wastewater" (APHA-AWWA-WEF, 1998). Recommendations for analysis methods and laboratory selection, including choosing a certified lab, are discussed in the SFAN Freshwater Quality Protocol Narrative.

2.0 TECHNIQUES

Tips for collection of bacteria samples:

- Collect water samples first before disturbing the sediment
- Note potential sources of contamination at each site
- Wear appropriate disposable, powderless gloves
- Use correct sample-handling procedures to avoid sample contamination
- Establish a routine for sample collection; use a consistent sampling technique
- Obtain training for and practice field techniques under supervision before collecting water samples.
- Collect a sufficient number of appropriate types of quality-control samples
- Prevent nose, mouth, eye, and direct skin contact with water

Aseptic Technique (from O'Ney, 2004)

Disposable latex or rubber gloves should be used to collect bacteria samples. Some individuals have severe allergic reactions to latex. Field staff must avoid touching the opening of the sample collection container or its cap, or having the sample touch hands or arms. For each sample:

- Wash and scrub hands thoroughly to the mid-forearm, using antibacterial hand soap (or a hand sanitizer at 50 ppm chlorine equivalency, if available).
- Open the sample container taking care to avoid touching the inside surfaces or otherwise causing contamination
- Remove a glove by holding it from the wrist side opening inner surface. Avoid any contact with the outer surface of the glove.
- Do not touch anything with the exterior of the glove except the sample.
- If you have concern that the glove may be contaminated, discard that glove and use another sterile glove.
- With the gloved hand, collect the sample.
- After sample has been collected, close the sample container, remove and discard the glove and

Sample Bottles

Use only a sterile 100 mL bacteriologic sample bottles (supplied by the laboratory). It is important to have extra bottles as they occasionally can be swept away in current or contaminated. Some laboratories provide bottles with relatively "waterproof" labels already attached. Other labels are more susceptible to wear and generally consist of regular paper. If this is the case, it is best to place a scotch tape over the label. It is sometimes easier and more efficient to label the bottle before sampling; this avoids having to dry off the bottle or write on a wet label. Pre-labeling (in the office or field vehicle) can also save time in the field especially important when it is raining.

Laboratory-supplied bottles may contain a tablet of sodium thiosulfate which is used to neutralize chlorine. This is required for drinking water samples. This tablet is not needed for SFAN surface water samples and does not affect the bacteria. However, in the event that chlorine may be present and to be consistent, the tablet should remain in the bottle.

Collecting the Samples
(Adapted from Wilde et al, 1999)

Prepare for sampling

1. Upon arrival at the field site, set out safety equipment such as traffic cones and signs.
2. Park vehicle in a location and direction to prevent sample contamination
3. Take extra bottles in case of contamination or loss
4. Take enough bottles to obtain QA/QC samples (see QAPP)
5. Label bottles but leave "time" field blank until actual sample collection

Determine the sampling location

1. Visually inspect the stream from bank to bank and longitudinally, observing velocity, width, and depth distribution, and apparent distribution of sediment and aquatic biota along the cross section. Note and document the location of stagnant water, eddies, backwater, reverse flows, areas of faster than normal flow, and piers or other features along the cross section.
2. Check the site list to determine whether the sample is to be collected in a pool or flowing area (or both). If sampling from a flowing area, identify the area of the stream that appears to be completely mixed (the centroid of flow). This may be determined ahead of time from reliable discharge measurements (see Initial Site Establishment – SOP#11 and Flow Measurement – SOP#9). Do not disturb the sediment before collecting a water sample.
3. For pools, if shallow (< 1 ft) take measurements at a middle depth. If the pool is from 1-4 ft deep collect a sample at a depth that meets monitoring objectives (Wilde et al, 1999).
4. For flowing water, sub-surface samples are taken at 0.1 m (4 inches) below the water surface if water level is < 5 ft (1.5 m). Samples are collected at the surface when water depth is < 0.1 m (Puckett, 2002). Sampling from the shoreline of any water body (meaning standing on shore and sampling from there) is the least acceptable method, but in some cases is necessary (Puckett, 2002).
5. Collect the bacteria sample at the same location as you will be collecting the core parameters.

Water samples should be collected from a location in the stream where the stream visually appears to be completely mixed. Ideally this would be at the centroid of the flow (*Centroid* is defined as the midpoint of that portion of the stream width, which contains 50% of the total flow), but depth and flow etc. do not always allow centroid collection (Puckett, 2002).

Collect the sample

Note: Collect water samples first to avoid disturbing the sediment and re-suspending sediment or bacteria

The USGS uses the "Hand-dip" method (Myers, 2003) if stream depth or velocity is not sufficient to use depth-integrated sampling. The procedure minimizes the collection of surface films and avoids contact with the streambed. The method is as follows:

1. Open a sterile, narrow-mouth borosilicate or plastic bottle; grasp the bottle near the base, with hand and arm on the downstream side of the bottle.
2. Without rinsing, plunge the bottle opening downward below the water surface. Allow the bottle to fill with the opening pointed slightly upward into the current.
3. Remove the bottle with the opening pointed upward toward the water surface and tightly cap it, allowing about 2.5 to 5 cm of headspace. Laboratory supplied bottles typically have an "EPA fill line" that allows for this amount of headspace.
4. Inspect each sample, looking for overfilling and (or) the presence of large amounts of particulates that might have been captured due to excessive streambed disturbance during sample collection. If you note either or both of these conditions, discard the sample, making sure there are no residual particulates left in the container, and resample.
5. Place the sample bottle in an ice-chest immediately. [NOTE: Use blue ice (often provided by labs), not wet ice to avoid possible contamination by contact with the melt water.] Ensure that the bottle label is completed with the date, time, site ID, and initials of field personnel.
6. Check the temperature of the ice-chest and refrigerator (if used); it must be between 1-4 °C. Samples should be stored in the dark.
7. Ensure that the samples are transported to the laboratory within the 6 hour EPA hold time.

3.0 FIELD PREPARATIONS AND LABORATORY COORDINATION

When starting work with a new laboratory, the Water Quality Specialist should develop a good working relationship with a laboratory manager and also a chemist/microbiologist at the laboratory. Discuss analytical methods, detection limits, holding times, and laboratory constraints such as limited incubator size. Obtain official chain of custody forms from the lab as well as any needed bottles, cooler, and ice packs if the laboratory provides these. Discuss sample drop-off and pick-up possibilities. Also discuss the labs' capacity for the number of samples you will have. General tasks list include:

- ♦ Notify the lab at the beginning of the season, or as early as possible, of your sampling schedule
- ♦ Call the lab the day before or the morning of sampling to verify sample collection
- ♦ If at all possible, schedule sampling early in the week rather than later.
- ♦ Fill out the chain of custody form ahead of time except for the sample time; include the dilution in the comments field*

* It is critical to know the expected concentrations of fecal coliforms since dilutions may be required in order to quantify bacteria (i.e., in order to avoid "censored data" that is greater than (>) or less than (<) a detection limit). Refer to the site list for dilution information.

The chain of custody form is a means of tracking samples from receipt in the laboratory through analysis, to final disposal of the sample. It should be filled out in ink. The chain-of-custody forms travel with the samples during the transfer, and are filed in the laboratory project files. Upon arrival at the laboratory, the "sample custodian" at the lab inspects the sample containers to ensure that the sample seals are intact and the sample containers have not been damaged. If any seals have been broken and/or any sample containers damaged, the sample custodian records the condition of the seals and containers on the chain-of-custody Form. The sample custodian takes custody of the samples by signing, dating, and noting the time in the on the chain-of-custody Form.

Once at the laboratory, if samples need to be subdivided and submitted to another laboratory sub-contractor, this information should be noted on the original Chain-of-Custody Form, and a new Chain-of-Custody Form with the other lab should be initiated (Puckett, 2002).

<u>Equipment Checklist</u>
Scotch tape
Hand sanitizer
Bottle labels
Disposable gloves
Sterile sample bottles
Data sheets printed on waterproof paper
Ice chest

Thermometer for ice chest
Chain-of-custody form
Sharpie (permanent pen)
Water jug for washing hands
Soap

Also, consult SOP#2 for safety equipment as well as SOP#3 for equipment calibration.

4.0 QUALITY ASSURANCE/QUALITY CONTROL (CHECK WITH QAPP)

"Depending on the data quality requirements of the study and site conditions, quality control samples (field blanks and field replicates) generally constitute from 5 to 20 percent or more of the total number of samples collected year a given period of time (Myers, 2003). A field duplicate and a field blank are required as follows:

Field Blank – 1 in every 10 to 20 samples; pass sterile buffered water (?) into a sterile sampling container; have container analyzed for FIB

Field Replicate – collect and analyze 1 field replicate for every 10-20 samples. A split sequential replicate is recommended. Two samples are collected and each sample is analyzed in duplicate (Myers, 2003).

5.0 REFERENCES

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